RESEARCH ARTICLE

Morin Hydrate Mediated Biosynthesis of Silver Nanoparticles and Its Antimicrobial Activity

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ABSTRACT

Morin hydrate is a flavonoid that is chosen as an active ingredient for synthesizing silver nanoparticles. In order to characterize the morin hydrate assisted silver nanoparticles (MHNP), field emission scanning electron microscopy (FE-SEM), ultraviolet-visible spectrophotometry, energy dispersive X-ray spectroscopy (EDAX), dynamic light scattering analyzer (DLS) was used, and inductively coupled plasma-optical emission spectroscopy (ICP-OES) studies were used to determine the total amount of silver contained in the liquid state of the MHNP. Then, the formed nanoparticles were evaluated for antimicrobial effect. In the UV-visible spectroscopy, maximum absorption was observed at 444.5 nm, which is characteristic of silver nanoparticles. According to FE-SEM data, the generated nanoparticles were spherical, and DLS analysis determined that the average particle size was 83.4 nm. EDAX images additionally proved that silver was present in the sample. The antibacterial studies revealed that the nanoparticles were effective only against certain gram-positive bacterial strains; therefore, they can be used in biomedical applications, especially for treating infections caused by gram-positive bacteria.

Keywords: Morin hydrate, Silver nanoparticles, Antimicrobial activity.

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INTRODUCTION

Several thousand years ago, Indian traditional medicine originated as one of the oldest and most intricate systems to treat diseases. This process involves using plants, minerals, and metals to create remedies for curing illnesses.[1] Nanotechnology is an advanced field of science that utilizes matter at an atomic and molecular level to create particles in the nano range. Today, nanotechnology has made remarkable progress and has led to the synthesis of various particles, such as nanowires, nanotubes, and nanoparticles. Different methods of producing metal nanoparticles include physical, chemical, and biosynthesis methods.[2] Nonetheless, physical and chemical methods are linked with various health risks and can be toxic to living organisms and the environment. Therefore, there is a need for a non-hazardous and eco-friendly method for producing metal nanoparticles.[3] Flavonoids are bioactive compounds that are derived naturally from plants and have a significant impact on human health. A bioflavonoid morin hydrate (Figure 1) is primarily extracted from the fruits, stems, and leaves of plants belonging to the Moraceae family. There is plenty of evidence to support the claim that morin hydrate exerts beneficial effects against various chronic degenerative diseases that can be life-threatening.[4] The use of flavonoids in plant materials to synthesize metal nanomaterials has proven to be both effective and environmentally friendly. Flavonoids act as reducing and electrostatic stabilizing agents, making them an ideal choice for "Green" synthesis. This innovative approach not only produces high-quality nanomaterials but also helps to reduce the impact on the environment.[5] Previous literature reported the dihydromyricetin-mediated silver nanoparticle synthesis and their efficacy against fungal pathogens tested *in-vitro*.[6] Recently, silver nanoparticles were synthesized from myricetin (MY), a dietary flavonoid, and evaluated for their antioxidant and antibacterial activities.[7] In this work, we report on the green production of silver nanoparticles mediated by morin hydrate and their characterization and assessment of antibacterial activity.

MATERIALS AND METHODS

Materials

Morin hydrate and silver nitrate were purchased from Sigma Aldrich India for this study. To ensure accuracy and precision

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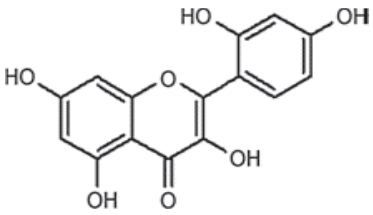


Figure 1: Structure of morin hydrate[4]

in our results, we used Milli-Q water exclusively throughout the entire study. We utilized only analytical-grade chemicals in our study.

Bacterial Strains

The antibacterial potential of MHNP was evaluated against *Bacillus cereus, Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi, Klebsiella pneumoniae*, *Shigella dysentriae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.

Biosynthesis of Morin Hydrate-Mediated Silver Nanoparticles

Several experiments were carried out in the morin hydrate-mediated silver nanoparticles (MHNP) synthesis process, and it was ultimately determined that an aqueous extract of morin hydrate could be produced by dissolving 25 mg of Morin hydrate in 50 mL of Millipore water. The solution should be warmed to make it soluble. Then, 40 ml of a filtered aqueous extract was treated with 60 mL of 1-mM silver nitrate solution in an iodine flask and stored for characterization studies, producing MHNP.

Characterization of MHNP

The preliminary characterization of MHNP was carried out by an ultraviolet-visible spectrophotometer (SHIMADZU UV-1800 240V) in the 300 to 800 nm wavelength range. Then, further, field emission scanning electron microscopy (FE-SEM) (ZEISS) with EDAX analyzed their size, shape and the presence of elemental silver in MHNP. A dynamic light scattering analyzer (DLS) analyzer (Horiba Scientific Nanopartica, nanoparticle analyser, SZ-100) determined size distribution and stability. ICP-OES (Perkin Elmer optima 5300 DV) was used to determine the amount of silver nanoparticles in the aqueous solution.

Screening Antibacterial Property of Synthesised Nanoparticles[8]

The biofabricated silver nanoparticles were tested for antibacterial activity at the different concentrations for nine pathogenic bacteria, including three-gram-positive (*B. subtilis*, *B. cereus*, and *S. aureus*) and six-gram negative bacteria (*E. coli*, *K. pneumoniae*, *S. typhi*, *S. dysentriae*, *P. mirabilis*, and *P. aeruginosa*) by using cup plate method. Sterilized water was used as control. Microbial cultures free from contaminants were transferred onto Muller-Hinton agar for further subculturing. A sterile cotton swab was used to spread the suspension on nutrient agar and left to dry for 10 minutes. Four 4 mm diameter wells were made per plate

Figure 2: Synthesis of MHNP with morin hydrate

at the agar surface using a sterile metal borer. In each well, varying concentrations of silver nanoparticle solution (25, 50, 75, and 100 µL) and a control were added under aseptic conditions. The plates were kept in a warm incubator at a temperature of 37°C for a period of 24 hours. The plates were kept in an incubator for a full day at 37°C. Subsequently, the millimetre-scale zone of inhibition for every concentration was recorded.[9]

RESULTS AND DISCUSSION

The sustainable synthesis of MHNP from silver nitrate solution with an aqueous extract of morin hydrate was identified by a color change from colorless to yellowish-brown after 24 hours (Figure 2).

UV-visible Spectroscopy

The biosynthesis of MHNP was monitored by means of UV-visible spectroscopy. It was visually observed that Ag^{+} was reduced to Ag^{0}, which was indicated by a change in color. Adding morin hydrate extract caused the colorless silver nitrate solution to change, turning from yellowish-brown to reddish-brown. The absorption spectrum of the MHNP indicated that the maximum absorption (λ_{max}) was observed at 444.5 nm, as shown in Figure 3. This result is in good correlation with earlier published findings and was attributed to the presence of silver nanoparticles. It was found that a longer reaction time increases peakintensity, as shown in Figure 4.

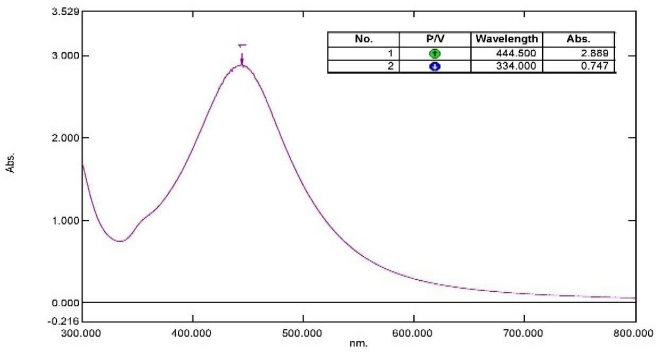


Figure 3: Ultraviolet-visible spectra of MHNP using morin hydrate

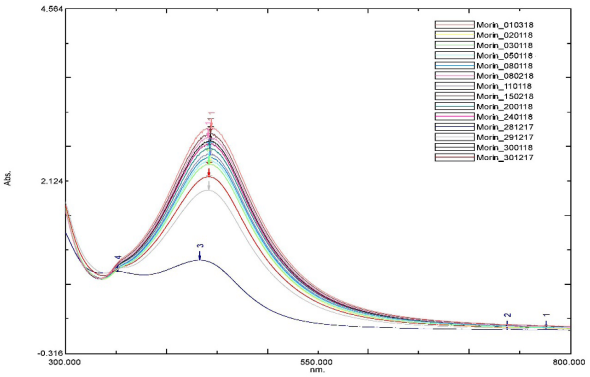


Figure 4: Ultraviolet-visible overlaid spectra recorded as a function of reaction time for synthesized MHNP using morin hydrate

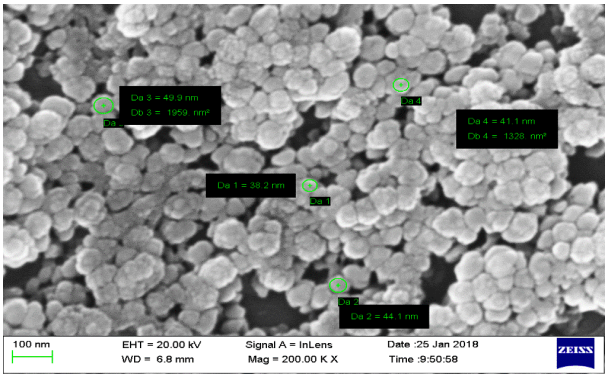


Figure 5a: FE-SEM micrograph of MHNP synthesised from morin hydrate

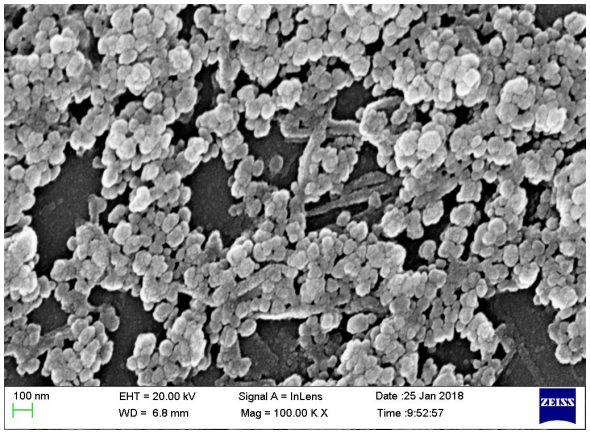


Figure 5b: FE-SEM micrograph of MHNP synthesized from morin hydrate showing spherical-shaped particles

FE-SEM and EDAX Studies

Surface morphology and elemental analysis of MHNP was accomplished by EDAX coupled with SEM, Oxford Instruments, UK. The MHNP were placed over the sticky carbon tape fixed on a microscopic stub of aluminum. FE-SEM provides the surface morphology of MHNP. The pattern of morin hydrate synthesized MHNP displayed that spherical-shaped nanoparticles were produced, and the size of the particles ranged from 38 to 50 nm on a 100 nm scale

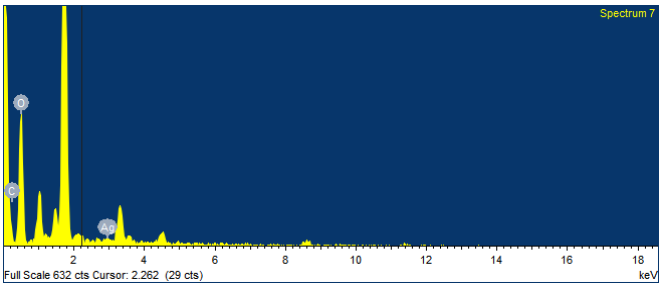


Figure 6: EDAX spectrum of MHNP synthesized using morin hydrate

(Figure 5a & 5b). Silver's presence was also confirmed using the EDAX spectrum (Figure 6). The EDAX image showed that the signal observed in the range between 2.7 and 3 keV,[10] and the signal for carbon, oxygen and other unidentified signals were also observed, indicating that morin hydrate may form a layer over silver nanoparticles.

DLS Analysis

DLS is used to measure the particle's molecular size less than a nanometer to several microns and also measures the zeta potential of the particles. Using DLS, the particle size was analyzed for the synthesized nanoparticles, and the total mean was found to be 83.4 nm, which means they are in the nano range (Figure 7). Also, the zeta potential was measured to express the degree of repulsion between similarly charged particles. High repulsion prevents agglomeration and increases the stability. The mean zeta potential of MHNP was found to be -48.0 mV (Figure 8); the negative charge of the formed silver nanoparticles indicates that they are more stable.

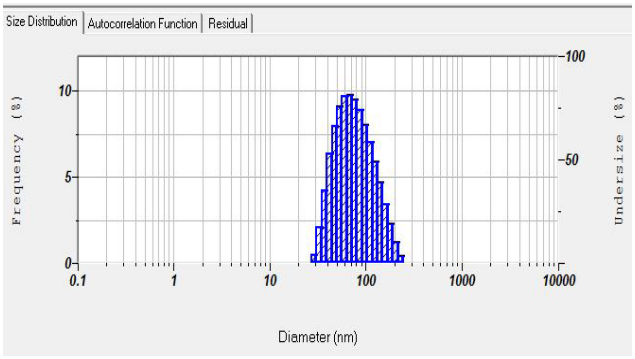


Figure 7: Particle size analysis of MHNP by DLS

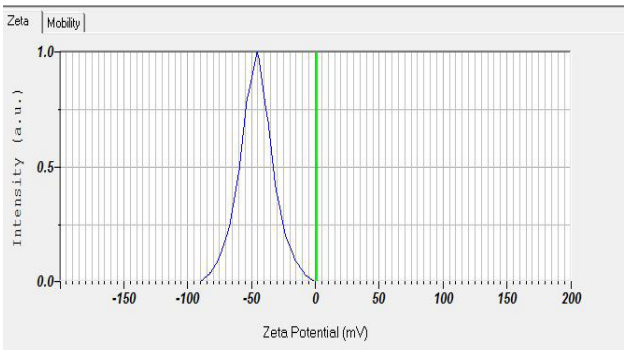
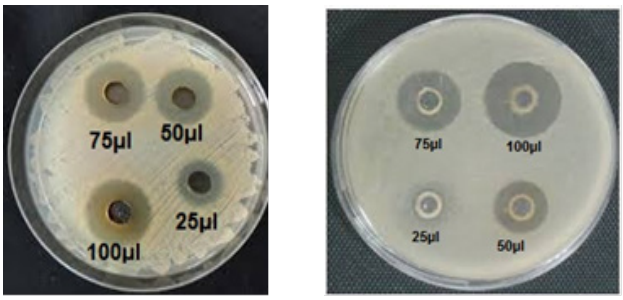


Figure 8: Zeta potential of MHNP

Table 1: Antimicrobial activity of MHNP

Test organisms	*Zone of inhibition in mm*			
	25 µL	*50 µL*	*75 µL*	*100 µL*
B. subtilis	12	15	17	22
B. cereus	-	13	15	20
S. aureus	-	-	-	-
E. coli	-	-	-	-
K. pneumoniae	-	-	-	-
S. typhi	-	-	-	-
S. dysentriae	-	-	-	-
P. mirabilis	-	-	-	-
P. aeruginosa	-	-	-	-



a. *B. subtilis* **b.** *B. cereus*

Figure 9: Antimicrobial activity of MHNP against a) *B. subtilis* b) *B. cereus* at 25, 50, 75 & 100 µL

ICP-OES Study

The concentration of silver nanoparticles synthesized in the aqueous medium was determined by ICP-OES analysis. In this study, the concentration of silver in MHNP was found to be 69.48 mg/L at a wavelength of 328.068 nm.

Antimicrobial Study

MHNP were studied for antimicrobial activity against several pathogenic microorganisms by well diffusion assay to identify the standard zone of inhibition (ZOI). The plates were incubated for 24 hours with 25, 50, 75 and 100 µL silver nanoparticles (Table 1). Due to the release of diffusible silver nanoparticles, bacterial growth was suppressed around the well. The antimicrobial activity results revealed that the formed silver nanoparticles were effective against selected gram-positive bacterial strains, and dose-dependent responses were also observed. However, gram-positive bacteria's cell wall is made up of a thick coating of peptidoglycan, which is made up of short peptides that cross-link linear polysaccharide chains. The formed morin hydrate-mediated silver nanoparticles could destroy this thick layer of the cell wall of gram-positive bacteria than gram-negative bacteria (Figure 9).

CONCLUSION

Though there are different methods of synthesizing silver nanoparticles, biological methods are the only way to compound silver nanoparticles in an easy, environmentally friendly, safe, sustainable, and highly effective way. There is currently a surge of interest in preparing and characterizing flavonoid-based nanomaterials using 'green' synthesis methods. In this work, we synthesized environment-friendly silver nanoparticles utilizing morin hydrate, and we assessed the antimicrobial potential of MHNP. Morin hydrate is a polyphenolic compound found in *Morus alba* L and red wine branches. It is widely distributed in the Moraceae family and in fruits such as almonds and sweet chestnuts.[11] Since isolating and purifying compounds from plants is a cumbersome and time-consuming process, morin hydrate was purchased commercially and used to synthesize silver nanoparticles. Study results suggested that the morin hydrate could have formed a layer over silver nanoparticles. As morin hydrate was already reported to possess various pharmacological activities when it was used to synthesize silver nanoparticles, it may have a synergistic effect.

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